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IN-VITRO INVESTIGTIONS OF SOME CHOSEN BOTANICALS AND BAU-BIOFUNGICIDE ON MYCELIAL DEVELOPMENT AND CONIDIAL GERMINATION OF Cercospora arachidicola AND Cercosporidium personatum

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Abstract— Endeavors have been made to survey some plant removes specifically, Lycopersicon esculentum, Tagetus patula, Achras sapota, Azadirachta indica, Datura metel, Cymbopogon citrates, Polyalthia longifolia, Allium sativum and Allium cepa in vitro for the administration of leaf spot (tikka) ailment of groundnut cultivar Dhaka-1 brought about by Cercospora arachidicola and Cercosporidium personatum. Results demonstrated that all the tried plant concentrates and BAU-Biofungicide smothered the development of mycelium and restraint of conidial germination of C. arachidicola and C. personatum. Among the medications, the leaf concentrates of L. esculentum demonstrated the best pursued by leaf concentrate of D. metel, A. indica and BAU-Biofungicide if there should be an occurrence of mycelial development and conidial germination. Other plant removes additionally had inhibitory impacts. In the event of conidial germination and germination hindrance, the least successful plant concentrate was C. citrates. Leaf concentrate of A. sapota was the least powerful if there should arise an occurrence of mycelial development.

Keywords— In-vitro, Botanicals, BAU-Biofungicide, Mycelial development and Conidia germination.

1. Introduction

Groundnut (Arachis hypogaea L.) is an important annual legume crop belonging to the family Fabaceae growing in many tropical and subtropical countries of the world (Wudiri and Fatoba, 1992). It is a multipurpose and highly nutritious crop containing oil, food and its foliage or haulm provides a valuable fodder for livestock. More than 40 fungal diseases attack groundnut (Jackson and Bell, 1969), but in Bangladesh, the crop is subjected to attack by twenty-one diseases (Talukder, 1974; Ahmed and Hossain, 1985). Among the diseases, early leaf spot caused by Cercospora arachidicola and late leaf spot caused by Cercosporidium personatum are the most devastating and economically important foliar fungal disease and major yield reducing factor of groundnut worldwide (Backman and Crawford, 1984; Khaleque, 1985). The early and late leaf spots of groundnut although caused by two fungal species, C. arachidicola and C. personatum, they are commonly referred together as Tikka disease. This disease results in early defoliation thereby affecting the pod formation (Worthington and Smith, 1973). Loss in pod yield due to the diseases was recorded as 70% in groundnut (Subrahmanyam et al., 1980). The yield loss was calculated in the groundnut variety Dhaka-1 due to early and late leaf spot (Tikka) by over 30-48% in Bangladesh (Hossain et al., 2005). In addition to direct yield loss, they hamper seed quality by reducing seed size and seed weight (Souta, 1912; Arthur, 1929) and oil content (Gupta et al., 1988). Severe attack of the disease resulting in heavy defoliations of groundnut leaves (Harrison, 1969). The disease can be controlled by developing resistant varieties, seed treatment with non-conventional chemicals (Maiti et al., 2005), spraying fungicides, influence by sowing times (Naidu and Vasanthi, 1995), by using indigenous medicinal plants and biological control means (Kishore and Pande, 2005). The most acceptable method for controlling this disease is cultivation of resistant variety but there was no absolute resistant variety in the world (Wynne et al., 1991).

Increasing concerns about environment hazards caused by excessive usage of chemical fungicide, necessitates the development of more economical and eco-friendly alternative components of disease management. Biological control represents a less cost, environmentally friendly natural and ecological approach for controlling diseases that reduces chemical inputs and their effect on environment. The main purpose of the study was to evaluate selected nine plant extracts for their in vitro antifungal activity against C. arachidicola and C. personatum.

2. Materials and Methods

Laboratory experiment was carried out at Disease Resistant Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh following Completely Randomized Design (CRD) with four replications for bioassay of nine botanical extracts, BAU-Biofungicide (Trichoderma based preparation) and Bavistin (Carbendazim) against mycelial growth of C. arachidicola and C. personatum. The treatments were also tested in the present experiment following cavity slide technique to find out their effect on conidial germination of C. arachidicola and C. personatum.

2.1 Preparation of plant extracts

Plant extracts were prepared from fresh leaves of tomato (Lycopersicon esculentum), marigold (Tagetus patula), sapota (Achras sapota), neem (Azadirachta indica), datura (black) (Datura metel), lemongrass (Cymbopogon citrates) and debdaru (Polyalthia longifolia) and bulb of garlic (Allium sativum) and onion (Allium cepa). The collected fresh leaf samples of different plants were washed in running tap water to make free from dust. The plant materials were cut into small pieces and extracts were prepared by grinding in a mortar and pestle followed by crashing plant parts in an electric blender with sterilized distilled water at different doses. The mixtures were filtered through linoleum cloth. The extracts were kept in conical flasks separately before use. Concentration (weight/volume) of tomato leaf, datura leaf (black) and sapota leaf was 20%, neem leaf, marigold leaf, garlic clove and onion bulb were 25%. Lemongrass, BAU-Biofungicide and Bavistin were use tested at 1:2, 2.5% and 0.1% concentration, respectively.

2.2 Bioassay of botanicals, BAU-Biofungicide and Bavistin on C. arachidicola and C. personatum

Carrot leaf extract agar medium was prepared and poured into 90 mm Petri plates at 20 ml/plate. After solidification, three 5 mm discs of the medium were scooped from three places maintaining equal distance of 4 cm from the centre using a sterilized disc cutter. One millimeter of each of plant extracts and suspensions of BAU-Biofungicide and Bavistin was put into each hole and the plates were stored overnight in refrigerator for diffusion of the test materials into the medium surrounding the hole. Next day, the plates were inoculated at the center with 6 mm blocks of 15 days old culture of C. arachidicola and C. personatum. Three plates (replications) were maintained for each material. Control plates did not receive any material. To prevent contamination, the plates were covered with the Para film and the plates were incubated at 24 ± 1OC.

The isolates of C. arachidicola and C. personatum were grown on carrot leaf extract in Petri plates for 12 days at room temperature (24 ± 1OC). The culture plates were kept under NUV light for 3 days for maximum production of conidia. Conidia were collected from the plates by scraping with a sterilized glass slide and conidial suspension was prepared in sterilized distilled water. The suspension was filtered through two-ply cheese cloth to remove mycelial fragments and lumps of agar. The concentration of conidia in suspension was adjusted to 2x104 per milliliter using a hemocytometer (Krishna et al., 2001).

Observation was made regularly to record the mycelial growth. After inoculation periods of 10, 13 and 15 days the linear growth of mycelial of C. arachidicola and C. personatum was measured (McKeen et al., 1986; Nene and Thaplial, 1993; Islam et al., 2001) and percent inhibition of growth was calculated using

the following formula as suggested by Sundar et al. (1995):

$$\text{Inhibition (\%)} = \frac{X-Y}{X} \times 100$$

Where,
X = Mean mycelial growth (radial) of pathogen in control plate
Y = Mean mycelial growth (radial) of pathogen in treatment

2.3 Growth inhibition of C. arachidicola and C. personatum.

To assay the antifungal activity of the botanical extracts, Bavistin and BAU-Biofungicide, 50µl of conidial suspension and equal volume of the suspension of test materials transferred to the well of each cavity slide and mixed thoroughly. Three single cavity slides (replications) were used for each treatment. All slides were kept in a humid chamber prepared by lining 90 mm diameter Petri dishes with wet tissue paper and incubated in the dark at 24± 1OC. The slides were directly observed under light microscope for conidial germination at 24, 48 and 72 hours after incubation. Immediately after inoculation, a drop of lacto phenol-cotton blue was added to each well to prevent further germination of conidia. Fifty conidia in a well were observed under a compound microscope and number of germinated and non-germinated conidia was counted. Percentage inhibition of conidial germination in each treatment was calculated from the formula:

Percentage inhibition = (number of conidia germination in control–number of conidia germination in treatment/ number of conidia germination in control ×100 (Krishna et al., 2001).

3. Results and Discussion

3.1 Effect of nine different selected botanicals, Bavistin and BAU-Biofungicide on mycelial growth of C. arachidicola and C. personatum

The efficacy of different nine selected botanicals, Bavistin and BAU-Biofungicide were evaluated against mean mycelial growth and growth inhibition of C. arachidicola and C. personatum at 10, 13, 15 days after inoculation (DAI). The results are presented in Table 1.

Table 1. *In-vitro* evaluation of nine different selected botanicals, Bavistin and BAU-Biofungicide on mycelial growth of *C. arachidicola* and *C. personatum*

Treatments	10 DAI		13 DAI		15 DAI	
	Mycelium growth (cm)	Growth inhibition (%)	Mycelium growth (cm)	Growth inhibition (%)	Mycelium growth (cm)	Growth inhibition (%)
Tagetus patula	3.9	41.9	4.5	50.0	4.7	47.8
Datura metel	1.3	80.8	1.5	83.3	1.6	81.9
Azadirachta indica	2.9	57.9	2.2	75.6	2.3	74.4
Lycopersicon esculentum	0.9	86.3	1.1	87.4	1.2	86.7
Allium sativum	4.2	38.0	4.6	49.3	4.6	48.6
Achras sapota	5.6	17.3	6.4	28.6	8.9	14.4
Polyalthia longifolia	2.5	62.6	3.9	56.7	4.4	51.4
Cymbopogon citrates	5.2	23.5	8.5	5.6	8.9	0.8
Allium cepa	5.3	21.3	5.9	34.1	6.0	33.8
BAU-Biofungicide	1.9	71.8	2.2	75.9	2.4	73.7
Bavistin	1.0	85.2	1.4	84.8	1.4	84.8
Control (untreated)	6.8	-	9.0	-	9.0	-
LSD(P ≥0.001)	1.04	-	3.24	-	3.42	-

Data represent the means of three replications

All treatments with botanicals, Bavistin and BAU-Biofungicide reduced mycelial growth on culture plates

significantly over control. At 10 days after inoculation (DAI), the mean mycelial growth varied from 0.9 to 6.8 cm, where maximum and minimum mycelial growths were recorded under control (plain water) and leaf extract of Lycopersicon esculentum, respectively. Radial colony diameter under Bavistin, leaf extracts of Datura metel and BAU-Biofungicide was statistically similar to that under leaf extract of Lycopersicon esculentum. Maximum of 86.3% growth inhibition over control was obtained with leaf extract of Lycopersicon esculentum followed by Bavistin, Datura metel, BAU-Biofungicide, leaf extract of Polyalthia longifolia and Azadirachta indica. Minimum of 17.3% growth inhibition was recorded under the treatment with leaf extract of Achras sapota leaf. At 13 and 15 DAI, trends in mean mycelial growth and growth inhibition were identical as recorded at 10 DAI (Table 1). The findings of this trial are in agreement with the results of Natarajan et al. (2005), Kishore and Pande (2005), Patni et al. (2005), Buckinghum (1993), Srivastava and Lal (1997).

3.2 Effect of nine different selected botanicals, Bavistin and BAU-Biofungicide on conidial germination of C. arachidicola and C. personatum

The comparative effects of different nine selected botanicals, Bavistin and BAU-Biofungicide on conidial germination of C. arachidicola and C. personatum are presented in Table 2. All the botanicals, Bavistin and BAU-Biofungicide significantly reduce germination of conidia of the pathogens in the well of cavity slides compared to control (only water) at 24, 48 and 72 hours after incubation (Table 2).

At 24 hours of incubation, conidial germination ranged 0.0 to 68.0%, where the lowest conidial germination was observed in leaf extract of Lycopersicon esculentum, which was followed by leaf extract of Datura metel, Bavistin and leaf extract of Azadirachta indica. The highest conidial germination was recorded in untreated control. The extracts of onion bulb, Tagetus patula, Achras sapota and Cymbopogon citrates showed statistically similar effect on conidial germination after 24 hours of incubation. Among the treatments, leaf extract of Lycopersicon esculentum showed maximum (100%) inhibition of conidial germination. All the botanical extracts and BAU-Biofungicide inhibited conidial germination over 80% except leaf extract of Tagetus patula, Cymbopogon citrates and bulb of Allium cepa. BAU-Biofungicide, Bavistin, leaf extract of Datura metel and Azadirachta indica showed statistically similar effect on the inhibition of conidial germination (Table 2).

Table 2. Effect of nine different selected botanicals, Bavistin and BAU-Biofungicide on conidial germination of C. arachidicola and C. personatum following cavity slide method

	24 hour		*48 hour*		*72 hour*	
Treatments	*Conidial germination (%)*	*Germination inhibition (%)*	*Conidial germination (%)*	*Germination inhibition (%)*	*Conidial germination (%)*	*Germination inhibition (%)*
Tagetus patula	*30.7*	*69.3*	*46.7*	*53.3*	*52.0*	*48.0*
Datura metel	*1.3*	*98.7*	*16.0*	*84.0*	*25.3*	*74.7*
Azadirachta indica	*9.3*	*90.7*	*24.0*	*76.0*	*29.3*	*70.7*
Lycopersicon esculentum	*0.0*	*100.0*	*1.3*	*98.7*	*8.0*	*92.0*
Allium sativum	*18.7*	*81.3*	*26.7*	*73.3*	*39.3*	*60.7*
Achras sapota	*29.3*	*70.7*	*25.3*	*74.7*	*36.0*	*64.0*
Polyalthia longifolia	*21.3*	*78.7*	*30.7*	*69.3*	*26.7*	*73.3*
Cymbopogon citrates	*36.0*	*64.0*	*56.0*	*44.0*	*57.3*	*42.7*
Allium cepa	*32.0*	*68.0*	*36.0*	*64.0*	*37.3*	*62.7*
BAU-Biofungicide	*14.7*	*85.3*	*28.0*	*72.0*	*26.7*	*73.3*
Bavistin	*9.3*	*90.7*	*16.0*	*84.0*	*17.3*	*82.7*
Control (untreated)	*68.0*	*32.0*	*100.0*	*0.0*	*100.0*	*0.0*
LSD(P ≥0.001)	*20.54*	*20.54*	*22.17*	*22.17*	*21.37*	*21.37*

Data represent the means of three replications

At 48 hours of incubation, significantly the highest conidial germination (100%) was recorded from control,

whereas the lowest germination was found in leaf extract of Lycopersicon esculentum followed by Datura metel and Bavistin. The germination of conidia was 24% in leaf extract of Azadirachta indica. Conidial germination in the suspension of BAU– Biofungicide, extracts of Allium sativum, Polyalthia longifolia, Achras sapota and Allium cepa was statistically similar. In case of inhibition of conidial germination, the highest per cent of inhibition was observed in leaf extract of Lycopersicon esculentum, whereas the lowest was found in plain water (control). At 72 hours of incubation, conidial germination was reduced significantly over control under all the treatments with plant extracts, Bavistin and BAU-Biofungicide. Conidial germination ranged 8.0 to 100.0%, while the lowest conidial germination was recorded in leaf extract of Lycopersicon esculentum followed by Bavistin, leaf extracts of Datura metel, BAU-Biofungicide, leaf extracts of Azadirachta indica and Polyalthia longifolia. The highest (100.0%) conidial germination was observed in plain water (control). The conidial germination inhibition ranged 0.0 to 92.0%, where maximum and minimum conidial germination inhibition was recorded from the wells containing leaf extract of Lycopersicon esculentum and water (control), respectively (Table 2). The findings of the present research work are in consonance with the findings of Natarajan et al. (2005), Kishore and Pande (2005), Abdulrahman and Alkhail (2005), Aage et al. (2003). Among the treatments, the most effective was the leaf extracts of Lycopersicon esculentum followed by Datura metel, BAU-Biofungicide, leaf extract of Azadirachta indica in case of mycelial growth and conidial germination. Other plant extracts also had inhibitory effects but not as much as the leaf extracts of Lycopersicon esculentum, Datura metel, BAU-Biofungicide, leaf extract of Azadirachta indica. Leaf extract of Achras sapota was the least effective against mycelial growth of C. arachidicola and C. personatum. In case of conidial germination and germination inhibition the least effective plant extract was Cymbopogon citrates. The potentials of these plant extracts for pathogen control have not been fully realized largely because the experiment was performed in vitro. However, their effectiveness in field condition could be of a potential advantage as it will help to determine the in vivo inhibitory effect of the botanicals and BAU-Biofungicide.

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